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# The range-wide mitochondrial lineage of *Natrix natrix scutata* (Pallas, 1771) presented in the northern Zagros Mountains

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## Abstract

*Natrix natrix* (Linnaeus, 1758) is a common species distributed from Central Europe to Central Asia. In this range, it forms four subspecies that include several mitochondrial evolutionary lineages. One of the lineages, the so-called mtDNA lineage 8, has a wide distribution from the Baltic area to Anatolia and Kazakhstan. In Anatolia, this lineage meets several others, however, their occurrence is unclear, especially in the south-eastern Türkiye where the species is uncommon. Obtaining one specimen from the poorly studied Hakkâri Province (Zagros part of Türkiye), we investigated its genetic affiliation (mitochondrial DNA) and basic morphology. The specimen represents a unique haplotype of the mtDNA lineage 8, closely related to populations from Georgia and northern and north-eastern Türkiye. It thus extends the occurrence of this mitochondrial lineage representing subspecies *Natrix natrix scutata* (Pallas, 1771) southward to the northern edge of the Zagros Mountains. Despite the phenotype polymorphism of this species, the morphological comparison also confirmed that selected characters are similar to other populations of the region.

## Key Words

Anatolia, Colubridae, distribution, morphology, mtDNA, Natricidae

## Introduction

The Hakkâri Province is the easternmost Türkiye territory lying between Iran and Iraq, on the north-western edge of the Zagros Mountains. The province is thus highly elevated and zoogeographically influenced by the Middle Eastern herpetofauna, e.g., recently recorded *Platyceps rhodorachis* (Jan in de Filippi, 1865) (Yılmaz et al. 2021). Due to the topography, the Hakkâri Province is herpetologically poorly explored compared to other provinces of eastern Türkiye (e.g., Bitlis or Van; Akman et al. 2018; Yıldız et al. 2021). However, the expected diversity of snakes seems to be comparable with surrounding areas (cf. Sindaco et al. 2013) and includes now 22 species (Baran et al. 2021; Yılmaz et al. 2021). Two species of the family Natricidae, *N. natrix* and *N. tessellata*, are distributed there, whereby the latter species seem to be more common (Yılmaz and Avcı pers. data). Details on their distribution and the abundance in the province are, however, rather unknown. The same is true for their genetic affiliations. It is expected that such a so-called "Turkey clade" (sensu Guicking et al. 2009) of *N. tessellata* is presented in south-eastern Türkiye. This clade was already found near Van Lake as well as south of the Caspian Sea in northern Iran (Asztalos et al. 2021)

which means it should have continuous distribution in the area. On the other hand, for *N. natrix* the situation is not so unambiguous. The phylogeographic pattern of this species is formed by five mitochondrial (mtDNA) lineages, i.e., 1, 2, 6, 7, 8 (sensu Kindler et al. 2013) presented in the area between central Anatolia and the Caspian Sea (see fig. 3 in Asztalos et al. 2021). They represent two subspecies, where lineage 7 corresponds to *N. natrix moreotica* (Bedriaga, 1882) and the other lineages to *N. n. scutata* (Pallas, 1771). However, the sampling effort of these mtDNA lineages comes from distant areas, i.e., several hundred kilometres away from south-eastern Türkiye. Thus, it does not allow better hypotheses about the genetic affiliation and possible colonization routes of these extreme eastern Türkiye populations of *N. natrix*.

## Materials and methods

On 16 June 2020, we found a subadult individual (female) of *N. natrix* near Yürekli Village-Yüksekova, Hakkâri Province, Türkiye (37.4329°N, 44.3735°E, 1863 m a. s. l.). The individual was caught while basking near a stream (Nehil Reeds). We fixed the specimen with a 96% ethanol injection and stored it in 70% ethanol. The specimen (Fig. 1) is now deposited in the Zoology Museum of the University of Hakkâri (HAKZM), Türkiye under the voucher number HAKZM 3/2020. A collection permit No. 21264211-288.04-E.1048399 was issued by the Turkish Ministry of Agriculture and Forestry for the project FM19BAP2, Hakkâri University. We took basic morphological data following Dowling's (1951) and compared them with available morphological data from the literature (Table 1) within the range of evolutionary lineage 8 sensu Asztalos et al. (2021). The metric measurements were taken using dial calipers with 0.01 mm precision. Colour photographs were taken from the freshly dead specimen (Fig. 1). The tissue of the specimen (liver) was stored in 96% ethanol and used for DNA analysis. We extracted genomic DNA using standard DNA isolation kits and amplified the partial ND4 gene plus adjacent DNA coding for tRNAs (tRNA-His, tRNA-Ser, tRNA-Leu) fragments following Kindler et al. (2013). The resulting sequence (866 bp) has been checked using BLAST. Based on the % similarity, the newly obtained sequence was assigned to mtDNA lineage 8. To identify the mtDNA haplotype of the Hakkâri specimen, previously characterized haplotypes (Kindler et al. 2017; Asztalos et al. 2021) were added resulting in an 866-bp-long alignment of 22 ND4+tRNAs sequences. A parsimony network was drawn using TCS 1.21 (Clement et al. 2000), with gaps coded as fifth character states and a connection limit of 50 steps. The QGIS Desktop 3.20.1 software (2021) was used to visualize the distribution of mtDNA 1, 2, 6, 7, and 8 of *N. natrix*.

**Table 1.** Pholidosis counts (left/right) and body measurements (left/right, in mm) of *Natrix natrix scutata* (HAKZM 3/2020) from the Hakkâri Province, Türkiye.

| | **This study** | **Orlov and Tuniyev (1992)** | **Ilgaz et al. (2005)** | **Göçmen et al. (2011)** | **Afrasiab et al. (2012)** | **Gordeev (2012)** | **Klenina (2020)** |
|---|---|---|---|---|---|---|---|
| **Sex** | Female | Both sexes | Female | Female | Both sexes | Females | Females |
| **Loreal (absent or present)** | present | – | – | – | – | – | – |
| **Preocular** | – | 1-1 | 1/1 | 1/1 | – | – | – |
| **Postocular** | 3/3 | 3-3 | 3/3 | 3/3 | 3 | 2-3 | – |
| **Temporal** | 1/1 | – | – | – | – | – | 1-1 |
| **Posttemporal** | 1/1 | – | – | – | – | – | 1–3 |
| **Supralabialia** | 7/7 | 7–8 | 8/8 | 8/8 | 7 | 6–7 | 6–7 |
| **Sublabialia** | 10/10 | 9–11 | 10/10 | 10/10 | 8–11 | 7–10 | – |
| **Dorsal+temporal (around Parietal)** | 8 | – | – | – | – | – | – |
| **Preventralia + Ventralia** | 1+176 | 164–178 | 163 | 164 | 173–180 | 173–186 | 159–181 |
| **Dorsalia (midbody)** | 19 | 17–20 | 19 | 20 | 19 | 16–19 | – |
| **Subcaudalia** | 81 | 52–79 | 65 | – | 60–69 | 55–62 | 50–64 |
| **Rostrum height** | 2.27 | – | – | – | – | – | – |
| **Rostrum width** | 4.61 | – | – | – | – | – | – |
| **Distance between nostrils** | 5.06 | – | – | – | – | – | – |
| **Eye Diameter** | 3.14 | – | – | – | – | – | – |
| **Loreal length** | 1.16/1.16 | – | – | – | – | – | – |
| **Pileus length** | 15.31 | – | – | – | – | – | – |
| **Pileus width** | 8.69 | – | – | – | – | – | – |
| **Pileus height** | 7.54 | – | – | – | – | – | – |


**Figure 1.** The specimen (HAKZM 3/2020) of *Natrix natrix scutata* from the Hakkâri Province, Türkiye. (**A**) Dorsal and (**B**) Ventral views of the body (**C–F**) Different views of the head.

## Results and discussion

The specimen HAKZM 3/2020 from the Hakkâri Province (Table 1, Fig. 1) represents the subspecies *N. n. scutata* (sensu Asztalos et al. 2021). The snout-vent length of the specimen is 484 mm, total length 632 mm. It has one large temporal borders postoculars posteriorly in each site. Supralabialia and sublabialia are 7/7 (left/right), 10/10 (left/right), respectively. Gordeev (2012) and Klenina (2020) stated that females from the Volganian Region in Russia have 6–7 supralabialia and 7–10 sublabialia, Ilgaz et al. (2005) and Göçmen et al. (2011) found 8-8 supralabialia and 10-10 sublabialia in Türkiye. Orlov and Tuniyev (1992) and Afrasiab et al. (2012) reported 7–8 supralabialia and 8–11 sublabialia in both sexes from the Caucasian region and Iraq. Surprisingly, the Hakkâri specimen has the highest number of subcaudal scales compared to published evidence (81 vs. 50–79). The number of ventral scales is within the range given in the literature (176 vs. 159–186; Table 1). The dorsum of the specimen is gray. Over this ground coloration there are black spots with the size of a scale. In addition, the ends of some scales are white, which is more evident in the middle of the body. The upper part of the head is usually gray-brown and yellow half-moons are clearly visible behind it. Black blotches are evident between the supralabial scales. The ventral side of the head is yellowish-white and without spots. The ground color of the venter is dirty white on the anterior and middle parts of the body, with black, small dots distributed along the later parts of the body. These dots begin to grow in the middle of the body and black dots dominate on ventral and subcaudal scales. The internasal band is discoloured with the form

of a sharp ridge between the first and second supralabials. The parietal band presents from the lower part of the eyes up to the contact of the 3rd and 4th supralabials. The upper part of 5th, 6th, and 7th supralabials are dark spotted. A collar band is present at the upper part of the head. The width and length of the collar band is formed by six and 15 dorsal scales. There is no contact with ventrals at the lower part of the head. The ground colour of the dorsum is yellowish-brown with 88 double rows of dark spots. The spottiness becomes a line at the end of the dorsum and on the tail. The ventral side is yellowish-white with small spots at the edge of the ventral plates (Fig. 1).

According to genetic data, the collected specimen clearly corresponds with haplotypes of mtDNA lineage 8 sensu Kindler et al. (2013, 2017) and Asztalos et al. (2021), particularly forms the unique haplotype "gn22" (GenBank accession number: OP555168). The genetically closest haplotypes (one mutation step) to this new haplotype are from Georgia (gn9, gn11, gn12), and northern and north-eastern Türkiye (gn9, gn14, gn19, gn21; Fig. 2A, B). The newly genotyped record represents an approximately 135 km extension of lineage 8 (Fig. 2A) to the south when comparing the southernmost locality of this lineage from Kayseri, Erciyes Mountain (18 km S Kayseri, 38.59°N, 35.51°E, ca 1,900 m a. s. l.; Asztalos et al. 2021), Türkiye. The previous nearest locality of lineage 8 to the Hakkâri locality is approximately distant 290 km, located in Armenia, Nshkhark (39.9742°N, 45.2392°E, 2,289 m a. s. l.). The distance of other genotyped localities and mtDNA lineages of the species is more than 385 km (Fig. 2).

Comparing published and here obtained data from the Hakkâri Province, lineage 8 is widely distributed from the Baltic states throughout the East European Plain to Kazakhstan, Crimea, Transcaucasia, central Anatolia, and the northern edge of the Zagros (Asztalos et al. 2021 and Fig. 2 of this study). Our records confirmed the extension to the proximity of mtDNA lineage 1 that is distributed in Iran and Azerbaijan. The presence of lineage 1 is not excluded in the Hakkâri Province and this question should be investigated in the future. The presence of mtDNA lineages 2 and 6 are also not excluded although we can expect that both prefer different environmental niches than lineage 8 due to their limited distribution range (Asztalos et al. 2021). It is evident that lineage 8 reaches high elevations, e.g., more than 2,200 m in Armenia (Jablonski et al. 2017) or 2,500 m in Türkiye (Van Province; Yıldız et al. 2021). The overall distribution of lineage 8 also suggests that it is probably focusing on colder environments in higher elevations or northern latitudes that allowed colonization of wide areas of the Western Palearctic. Thus, only further dense sampling may confirm possible contact of different mtDNA lineages in the region that could be limited by specific environmental conditions. As consequence, mtDNA lineage 8 is thus the most widely distributed lineage of *N. natrix* (cf. Asztalos et al. 2021), with possible distribution further south in the Zagros Mountains of Iraq and Iran.

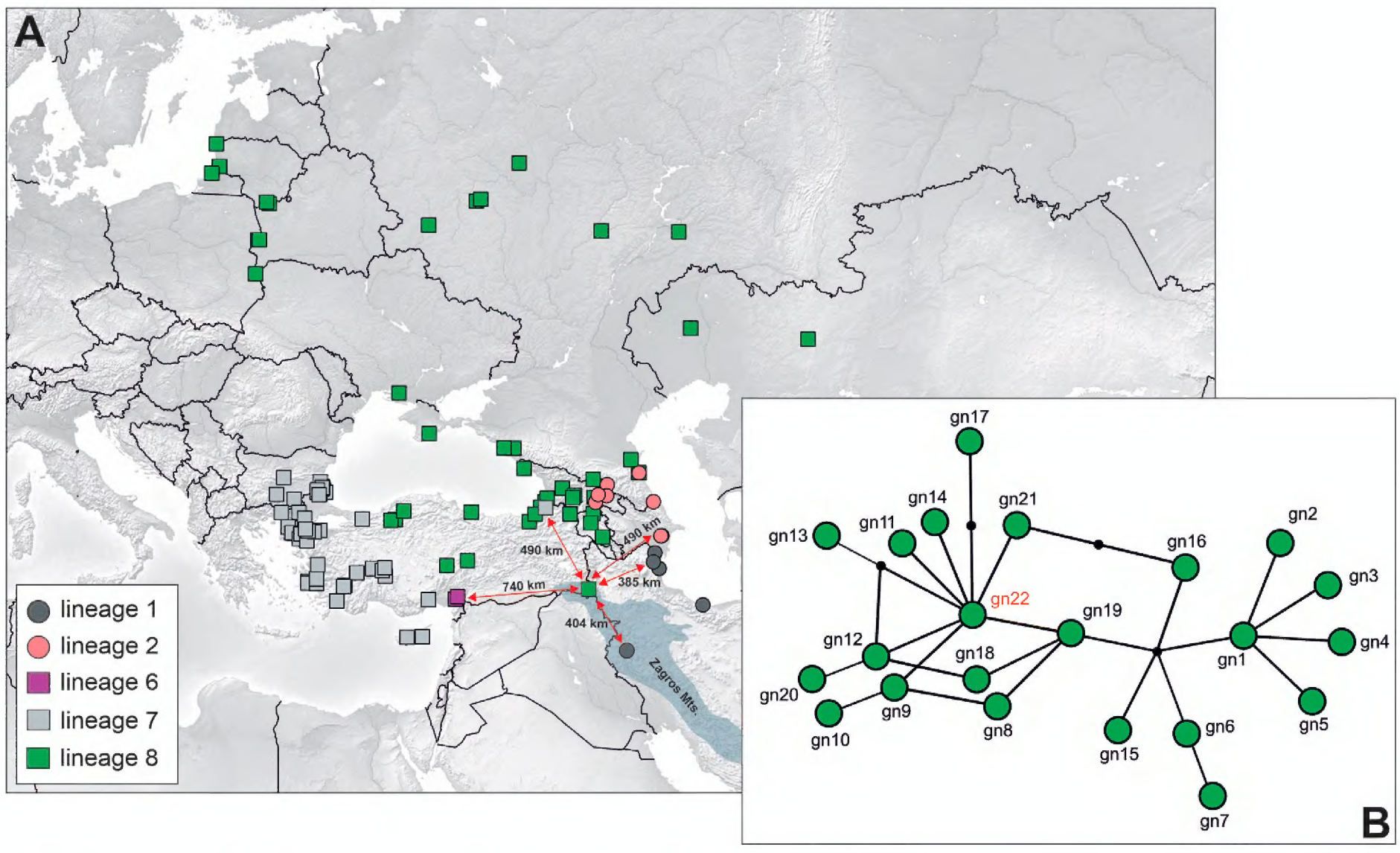


**Figure 2.** (**A**) Distribution of mtDNA lineages of *Natrix natrix* in Türkiye and surrounding areas based on Asztalos et al. (2021) and their geographic (air) distances to the record from the Hakkâri Province. (**B**) Parsimony network for 22 ND4-tRNAs sequences of mtDNA lineage 8. Symbol sizes reflect haplotype frequencies. Small black circles are missing node haplotypes; each line connecting two haplotypes corresponds to one mutation step. The haplotype affiliation of the Hakkâri specimen (HAKZM 3/2020) is marked in red (haplotype "gn22").

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